CLAIMS

1. A method and kit for determining the presence of bacteria or fungus-yeast ribonucleic acid (RNA) in a sample suspected of containing said bacteria and/or fungus, wherein said polynucleotide comprises a selected target region, said method comprising:

(a) extract bacteria or fungus-yeast ribonucleic acid (RNA) from the sample up to 1000 ml by centrifiltration on membranes and /or DEAE resin following by incubation with DNAse.

(b) incubating the bacteria or fungus-yeast ribonucleic acid (RNA) with a thermostable enzyme with RNA-dependent Reverse Transcriptase activity and with DNA-dependent Polymerase activity, allowing the combination of RT and PCR in a single-tube reaction, such as Tth DNA polymerase, and polynucleotide primers with a nucleotide sequence selected from the group consisting of

Seq ID No 2	TGCGGGACTTAACCCAACA	[primer reverse]
Seq ID No 4	TTACCCCACCTACTAGCTAAT	[primer reverse]
Seq ID No 6	TTGCGCTCGTTRCGGGACTT	[primer reverse]
Seq ID No 8	CGTTATCGCAATTAAGCAGACA	[primer reverse]
Seq ID No 10	TTGGGTAATTTGCGCGCCTG	[primer reverse]

under conditions which allow hybridization of the polynucleotide to the ribonucleotide target region and Reverse Transcriptase activity of said DNA polymerase for cDNA synthesis; and

(c) amplified the cDNAs formed to a detectable level by Polymerase Chain Reaction with said DNA polymerase and polynucleotide primers and probes with a nucleotide sequence selected from the group consisting of

Seq ID No 1	TGGAGCATGTGGTTTAATTCGA	[primer forward]
Seq ID No 2	TGCGGGACTTAACCCAACA	[primer reverse]
Seq ID No 3	AGAGTTTGATCATGGCTCAGA	[primer forward]
Seq ID No 4	TTACCCCACCTACTAGCTAAT	[primer reverse]
Seq ID No 5	GYGGAGCATGTGGYTTAATTCG	[primer forward]
Seq ID No 6	TTGCGCTCGTTRCGGGACTT	[primer reverse]
Seq ID No 7	GGGAAACTCACCAGGTCCA	[primer forward]
Seq ID No 8	CGTTATCGCAATTAAGCAGACA	[primer reverse]
Seq ID No 9	GGTAACGGGGAATWAGGGTTC	[primer forward]
Seq ID No 10	TTGGGTAATTTGCGCGCCTG	[primer reverse]
Seq ID No 11	TGCATGGYTGTCGTCAGCTCGTG	[probe forward]
Seq ID No 12	GAGTGGCGGACGGGTGAGTAA	[probe forward]
Seq ID No 13	ACAGGTGGTGCATGGTTGTC	[probe forward]
Seq ID No 14	TCAGCTCGTGTCGTGAGATGTT	[probe forward]
Seq ID No 15	ACAGGTGCTGCATGGCTGTC	[probe forward]
Seq ID No 16	TCAGCTCGTGTTGTGAAATGTT	[probe forward]
Seq ID No 17	AGGATTGACAGATTGAGAGCTCTT	[probe forward]
Seq ID No 18	CGGAGAGGGAGCCTGAGAA	[probe forward]
Seq ID No 19	CGGCTACCACATCCAAGGAA	[probe forward]

2. The method and kit of claim 1, wherein the cDNA target sequence synthetised by Reverse Transcriptase activity of the enzyme like Tth polymerase is amplified by the DNA-dependent Polymerase activity of DNA polymerase in the same tube by means of one step real time RT-PCR.

3. The method and kit of claim 1 and 2, wherein the composition for detecting bacteria comprising a polynucleotide primers and a probe consisting of the sequence

Seq ID No 1 TGGAGCATGTGGTTTAATTCGA [primer forward]
Seq ID No 2 TGCGGGACTTAACCCAACA [primer reverse]
Seq ID No 11 TGCATGGYTGTCGTCAGCTCGTG [probe forward]

4. The method and kit of claim 1 and 2, wherein the composition for detecting bacteria comprising a polynucleotide primers and a probe consisting of the sequence

Seq ID No 3 AGAGTTTGATCATGGCTCAGA [primer forward]
Seq ID No 4 TTACCCCACCTACTAGCTAAT [primer reverse]
Seq ID No 12 GAGTGGCGGACGGGTGAGTAA [probe forward]

5. The method and kit of claim 1 and 2, wherein the composition for detecting bacteria comprising a polynucleotide primers and a probe consisting of the sequence

Seq ID No 5 GYGGAGCATGTGGYTTAATTCG [primer forward]
Seq ID No 6 TTGCGCTCGTTRCGGGACTT [primer reverse]
Seq ID No 13 ACAGGTGGTGCATGGTTGTC [probe forward]
Seq ID No 14 TCAGCTCGTGTCGTGAGATGTT [probe forward]
Seq ID No 15 ACAGGTGCTGCATGGCTGTC [probe forward]
Seq ID No 16 TCAGCTCGTGTTGTGAAATGTT [probe forward]

6. The method and kit of claim 1 and 2, wherein the composition for detecting fungus-yeast comprising a polynucleotide primers and a probe consisting of the sequence

Seq ID No 7 GGGAAACTCACCAGGTCCA [primer forward]
Seq ID No 8 CGTTATCGCAATTAAGCAGACA [primer reverse]
Seq ID No 17 AGGATTGACAGATTGAGAGCTCTT [probe forward]

7. The method and kit of claim 1 and 2, wherein the composition for detecting fungus-yeast comprising a polynucleotide primers and a probe consisting of the sequence

Seq ID No 9	GGTAACGGGGAATWAGGGTTC	[primer forward]
Seq ID No 10	TTGGGTAATTTGCGCGCCTG	[primer reverse]
Seq ID No 18	CGGAGAGGGAGCCTGAGAA	[probe forward]
Seq ID No 19	CGGCTACCACATCCAAGGAA	[probe forward]

8. The method and kit of one of claims 1 to 6, wherein the preferred combination of primers and probes used for detection all bacteria and/or fungus-yeast consisting of the sequence :

Seq ID No 1+ Seq ID No 2 +Seq ID No 11

or

Seq ID No 3+ Seq ID No 4 +Seq ID No 12

or

Seq ID No 5+ Seq ID No 6 +Seq ID No 13 + Seq ID No 14 + Seq ID No 15 +Seq ID No 16

or

Seq ID No 7+ Seq ID No 8 +Seq ID No 17

or

Seq ID No 9+ Seq ID No 10 +Seq ID No 18 + Seq ID No 19

or

Seq ID No 1+ Seq ID No 2 +Seq ID No 11 + Seq ID No 7+ Seq ID No 8 +Seq ID No 17

or

Seq ID No 3+ Seq ID No 4 +Seq ID No 12 + Seq ID No 7+ Seq ID No 8 +Seq ID No 17

or

Seq ID No 5+ Seq ID No 6 +Seq ID No 13 + Seq ID No 14 + Seq ID No 15 +Seq ID No 16 + Seq ID No 9+ Seq ID No 10 +Seq ID No 18 + Seq ID No 19

9. The method and kit of one of claims 1 to 8, wherein the polynucleotide primers and probes are natural nucleic acid or Peptide Nucleic Acid (PNA) which can hybridize to nucleic acid (DNA and RNA).

10. The method and kit of one of claims 1 to 9, and also quantified this RNA for a comparison with quantified external standard RNA from by exemple *Escherichia coli* and *Candida spp.*